Diet and Reproductive Biology of the Rocky Intertidal Prosobranch Gastropod *Tricolia pulloides*

BY

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(5 Text figures)

INTRODUCTION

ALTHOUGH *Tricolia pulloides* (Carpenter, 1865) is a common inhabitant of the intertidal region on California rocky shores, it is small and often overlooked. Very little is known of its natural history. A related European species, *Tricolia pullus* (Linnaeus, 1758), has been studied extensively; *T. pullus* feeds on diatoms (FRETTER & GRAHAM, 1962). Females shed their eggs singly into the water. Development is pelagic, and the embryos, unlike those of many prosobranchs, pass through a free-swimming trochophore stage (LEBOUR, 1937; FRETTER, 1955). A related Indo-Pacific form, *Tricolia* (formerly *Hiloa*) *variabilis* (Pease, 1861) has been studied in Hawaiian inshore waters where it is abundant. The species is sexually dimorphic. The eggs, often laid on the brown alga *Padina,* exhibit direct development and hatch as juvenile snails, thus omitting a pelagic larval phase (WERTZBERGER, 1968a, b; KAY, 1979).

Studies of *Tricolia pulloides* were conducted on the population in the midtide zone dominated by the red algae *Rhodoglossum affine* (Harv.) Kyl. and *Gigartina papillata* (C. Ag.) J. Ag. at Mussel Point, Pacific Grove, California, in the period April 1979 to June 1980. One objective was to examine the food and feeding habits of the snails, answering the questions: What are they eating? Where are they obtaining food? When are they eating? The second objective was to characterize reproduction and development in the species through studies of the annual cycle of reproduction and growth, the nature of the egg masses deposited by females, and the gross sequence and timing of events in embryonic development.

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FOOD STUDIES

Materials & Methods: To determine what *Tricolia pulloides* was eating and where the food was obtained, 5 snails were collected from each of 5 species of red algae: *Gigartina papillata, Gigartina leptorhynchos* J. Ag., *Gastroclonium coulteri* (Harv.) Kyl., *Rhodoglossum affine,* and *Cryptosiphonia woodii* (J. Ag.) J. Ag. All collections were made at or below the 1.5 ft. (45 cm) tidal level. The larger, more conspicuous snails were collected, without scrutinizing the algae for juvenile individuals. Each sample was placed directly in 5% formalin. The stomach of each snail was dissected out, and the contents placed on a slide in a drop of 30% corn syrup under a coverslip for viewing under a compound microscope. Stomach contents were identified with the assistance of Dr. Isabella Abbott. The large pennate diatoms present in the preparations were counted; the relative abundance of other materials was estimated.

To determine when *Tricolia pulloides* was eating over the tidal cycle, hourly samples, each of 5 snails, were collected from a horizontal rock face covered with *Rhodoglossum affine* about 0.5 ft. (15 cm) above zero tide level. Samples were taken over a 7 hour period. The samples were preserved, and gut contents analyzed as before.

Results: Between 90 and 95% of the contents of stomachs of snails from all 5 types of algae consisted of pennate diatoms. Five to ten percent of the gut contents consisted of small sponge spicules, unidentifiable organic matter termed detritus, and small pieces of epiphytic algae including coccoid blue-greens, *Dermocarpa* sp., *Collinsiella tuberculata* S. & G., and Florideophycids. No one of these categories normally comprised more than 1 - 2% of the total diet. The diatoms and other algae in the gut were browsed as epiphytes on the surfaces of macroalgae. *Tri-*

colia pulloides was never found feeding on encrusting algae, rocks, or exposed surfaces.

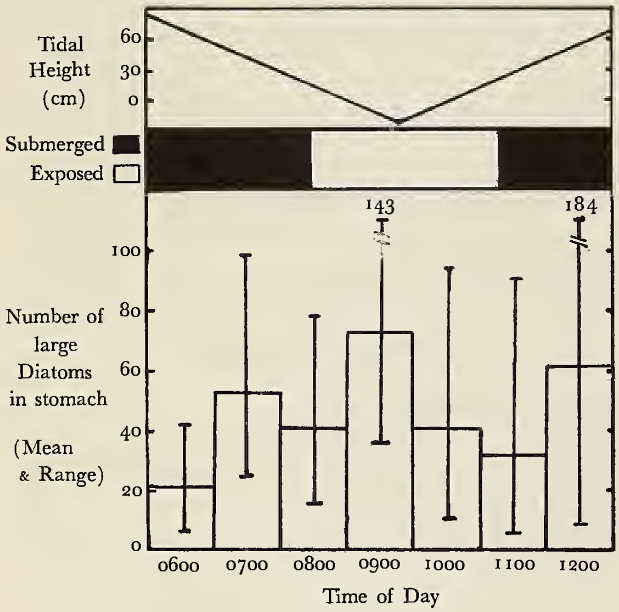

Figure 1

Number of large Diatoms in the stomachs of *Tricolia pulloides* at different phases of the tidal cycle

Figure 1 shows the results of sampling the gut contents of the *Tricolia* population over a 7 hour period centering on low tide. Quantity of food in the gut is shown in terms of number of large diatoms counted in squashes of the stomachs of the 5 snails sampled each hour. The population showed great variability, and the differences between sample means are not significant, but diatoms were most abundant in the stomachs at low tide.

Discussion: The diet studies show that *Tricolia pulloides* skims the surface of macroalgae, taking very largely pennate diatoms. Chun (1979) has noted large populations of sessile pennate diatoms on ungrazed, distal areas of fronds of *Rhodoglossum affine,* and noted a significant reduction in these populations through grazing by *T. pulloides* and other small herbivorous gastropods. Further, when submerged at high tide, *T. pulloides* tends to move out to these distal areas of algal fronds (Foster, 1979). The 5-10% of the diet not consisting of diatoms can probably be considered incidental. Particulate matter and epiphytic algae also occur in the areas browsed for diatoms and may be rasped up unintentionally. No single component comprises a large portion of the incidentals.

REPRODUCTIVE BIOLOGY

Materials & Methods: The following data were recorded for all snails collected: shell length from tip of spire to base of aperture, sex, and (for females) the number of mature eggs within the ovary. Sex was determined by cracking the shell in a small turnbuckle, removing the shell, and examining the gonads under a dissecting microscope. In females the gonad is translucent and bears large yellow eggs of uniform size. In males, the gonads are mottled and white.

To determine the annual reproductive cycle of *Tricolia pulloides,* monthly samples of *Gigartina papillata* and *Rhodoglossum affine* were taken from the same rocky area at the 0-1.5 ft. (0-45 cm) tide level at Mussel Point and were examined carefully for gastropods. All *T. pulloides* on fronds and holdfasts were removed and preserved in 5% formalin prior to dissection. The algal fronds were also checked for egg clutches of *T. pulloides.*

The egg masses of *Tricolia pulloides* were first identified as such by noting the great similarity between the large eggs in the ovaries of ripe females and the large eggs in certain egg masses commonly found on plants inhabited by *T. pulloides.* Laboratory observations of egg-laying and hatching confirmed the identification.

Yellow egg clutches were collected in the field from areas of dense *Tricolia pulloides* populations. Record was kept of the location of the egg masses on the algal frond, the type of alga bearing the egg mass, the diameter of the egg mass, and the number of eggs contained within the egg mass. The algal fronds bearing masses were then maintained in 12.5 cm diameter fingerbowls of seawater at about 14° C. The water was changed twice daily, and the samples were checked daily under a dissecting microscope for stage of development.

To see if *Tricolia pulloides* would lay egg clutches consistently under laboratory conditions, 2 fingerbowls of 25 snails each were kept with 6-8 clumps of *Rhodoglossum affine* free of any previous egg clutches and other organisms, at about 14° C. Daily the water was changed and the *R. affine* fronds were checked. All new egg masses found were measured and the eggs were counted. Each clutch was then trimmed from the algal frond, placed in a depression slide with a coverslip, examined under a compound microscope, and the time and stage of development noted. These egg masses were then put in fresh seawater in fingerbowls, kept at 14° C, and the seawater changed twice daily. Five selected clutches were checked microscopically twice daily for general developmental features, and three other clutches were checked every half hour each day up to the time of hatching, to establish the time scale of development under these laboratory conditions.

Hatchlings from these clutches were kept on fronds of *Rhodoglossum affine* under the same laboratory conditions as those used for the observation of clutches. The diet of the hatchlings was determined by lightly squashing the bodies of five-day-old snails in 1 drop of 30% corn syrup between a slide and coverslip and viewing them under a compound microscope.

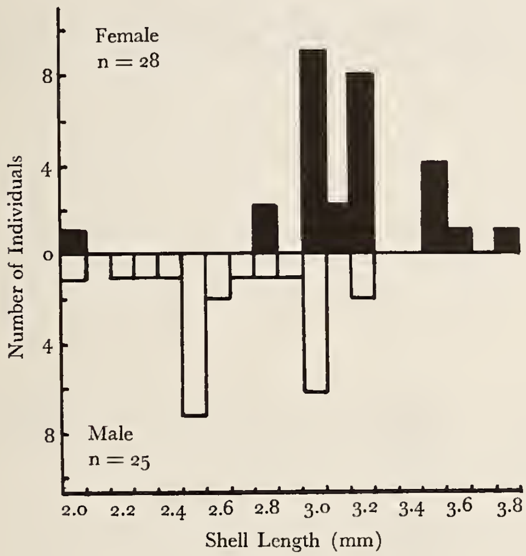

Figure 2

Size-class distribution of females and males of *Tricolia pulloides* in May 1979

Results: Figure 2 shows a distribution by size and sex of the 53 individuals 2.0 mm or more in shell length that were taken for diet studies (juvenile animals less than 2.0 mm long were not adequately represented in the sample). In this May 1979 sampling, males and females occurred in approximately equal numbers. Female snails averaged significantly larger than the males ($p < 0.001$); the mean shell length for females was 3.14 mm ($n = 28$), and for males was 2.62 mm ($n = 25$).

In the yearly cycle of the population (Figure 5), ripe females appear first in March. Examination of algal fronds shows that egg clutches are deposited only in May, June, and July. Thereafter the larger snails disappear from the population, leaving the small snails as a new year-class.

The eggs appear to mature rapidly and simultaneously in the ovary, for all individuals found either lacked enlarged ova or had full-sized eggs. The number of enlarged eggs counted in the ovary of individual females ranged from none (in one case) to 206, with a mean of 56.5 for 30 females (Figure 3). The number of eggs in an ovary tends to increase with the size of the female, but the variability at any particular shell length is great.

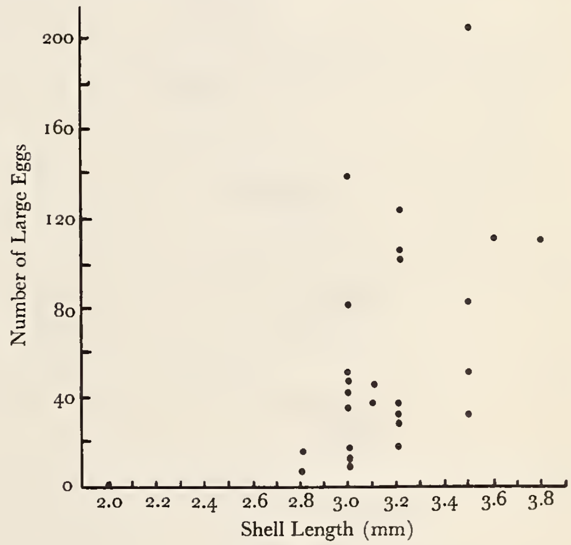

Figure 3

Relation between size (shell length) and number of large eggs in the ovary of female *Tricolia pulloides*

Fifty snails kept in fingerbowls in the laboratory at 14° C laid 57 egg clutches over a 6 day period. The number of eggs per clutch ranged from 11 to 104 with a mean of 32.5. These eggs in newly laid clutches appeared identical to those in the ovaries of mature female *Tricolia pulloides*. These eggs are light yellow, opaque, show a gradient in distribution of yolk, and each is enclosed in a colorless transparent capsule. The eggs and egg capsules are approximately 150 μm and 180 μm in diameter, respectively. Generally, the eggs are laid in a compact, sinuous ribbon which is coated with a clear jelly. The completed egg clutches are disc-shaped, average about 2.0 x 2.5 mm in diameter, and are attached by one flat surface to a plant. They are most generally found on a concave surface at the bifurcation of a frond on such red algal species as *Rhodoglossum affine, Gigartina papillata,* and *Gigartina leptorhynchos,* but some are found on *Cryptosiphonia woodii* which does not have a concave surface.

The time schedule of development, based on the external morphology of the embryo or larva, is shown in Figure 4. Zero time is the time the egg clutch was deposited; the time of fertilization is unknown. The first 6 cleavage stages each lasted from 0.5 to 1 hour. A few eggs showed abortive development; they became white and took on a granulated appearance after a few hours. By 24 hours the larval shell appeared complete. At about this time the tissues at the posterior end of the shell began to turn light green. The soft body parts pulled away from the interior of the shell antero-dorsally by 1.8 days, and soon portions are seen extending from the aperture. Pedal cilia and the oper-

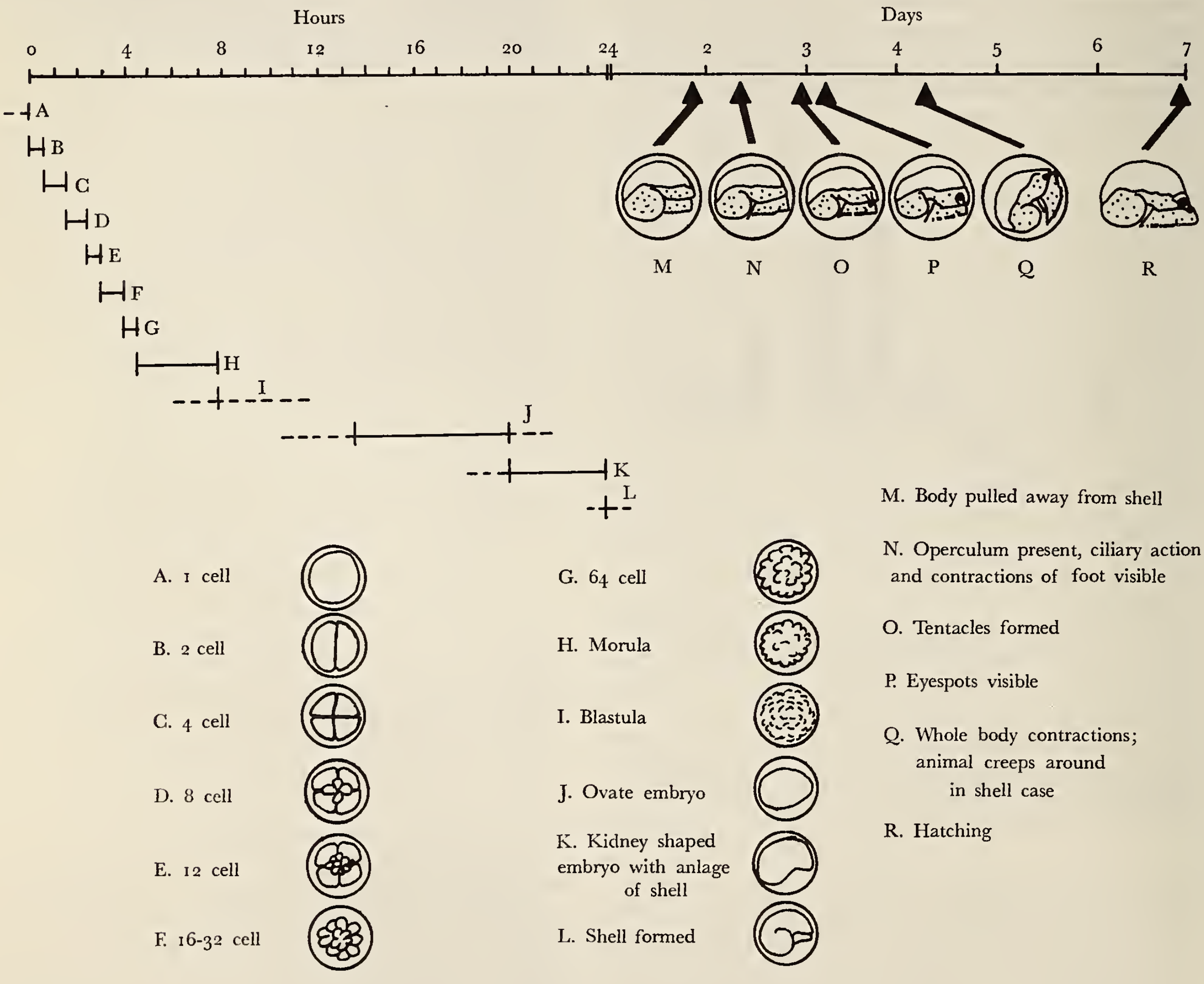

Figure 4

Average rate of development of *Tricolia pulloides* at 14°C. Solid bars show beginning and end of a stage. Bars ending in dotted lines indicate the approximate times certain features occurred whose exact beginnings and ends could not be determined accurately culum were formed by 2.3 days. Within 2 hours muscular contraction of the foot was seen. By 3.1 days the tentacles were present, and shortly thereafter black eyespots appeared. Contraction of the body into the shell and closure of the operculum were seen at 4.3 days. Soon the embryos began occasional rotations, crawling on the interior of the transparent egg capsule. Frequency of rotation increased just prior to hatching, and the jelly coating began to dissolve away about this time. At 7 days the embryos hatched as miniature young snails with nearly clear, planospiral shells, and with light green pigment concentrated in the area of the visceral hump. The maximum diameter of the shell at hatching was 240 μm.

After hatching, the hatchlings move out on the algal frond where they cling tenaciously from the very start. They are not swept away by a jet of water forcefully extruded from an eyedropper pipette. Snails knocked over maintain position by a mucus thread extruded by the foot. Whole mounts of 5-day-old snails crushed under a coverslip show that the young are eating primarily small, naviculoid diatoms. By 5 days after hatching, the shell aperture has widened with new shell growth. The body tissues are still relatively clear, except for the posterior parts of the visceral hump which are yellow and darker green. New shell is more darkly tinted and translucent. These juvenile snails were commonly found in the sandy holdfasts of algae in the midtide zone.

Discussion: Egg-laying in *Tricolia pulloides* occurs from May through July. From August 1979 to February 1980 snails bearing eggs were not seen (Figure 5). Snails less than 0.5 mm in length are very difficult to sex because of their small size, but even among larger snails, animals with eggs were unexpectedly scarce in the population sampled in 1980. This might be due to a sex ratio deviating from 1 : 1 or to a differential distribution of males and females in the habitat until late in the reproductive cycle, or possibly to protandric hermaphroditism. Finally, perhaps the sexes are indistinguishable by macroscopic features until near sexual maturity at 9 - 11 months of age, when eggs rapidly enlarge in the ovaries of large females. I am unable to evaluate these possibilities.

Since the ovary in most of the females examined contained more eggs than occurred in the average clutch (32), and great variability exists in the number of large eggs in females of a particular length, it seems likely that a female does not put all her eggs in one basket, so to speak, but instead lays two or more clutches for a given group of mature oocytes.

Tricolia pulloides has many features suiting it for laboratory studies. Egg clutches are easily obtained in the laboratory. No special conditions were required to stimulate egg-laying. The egg is large and yolky, and early

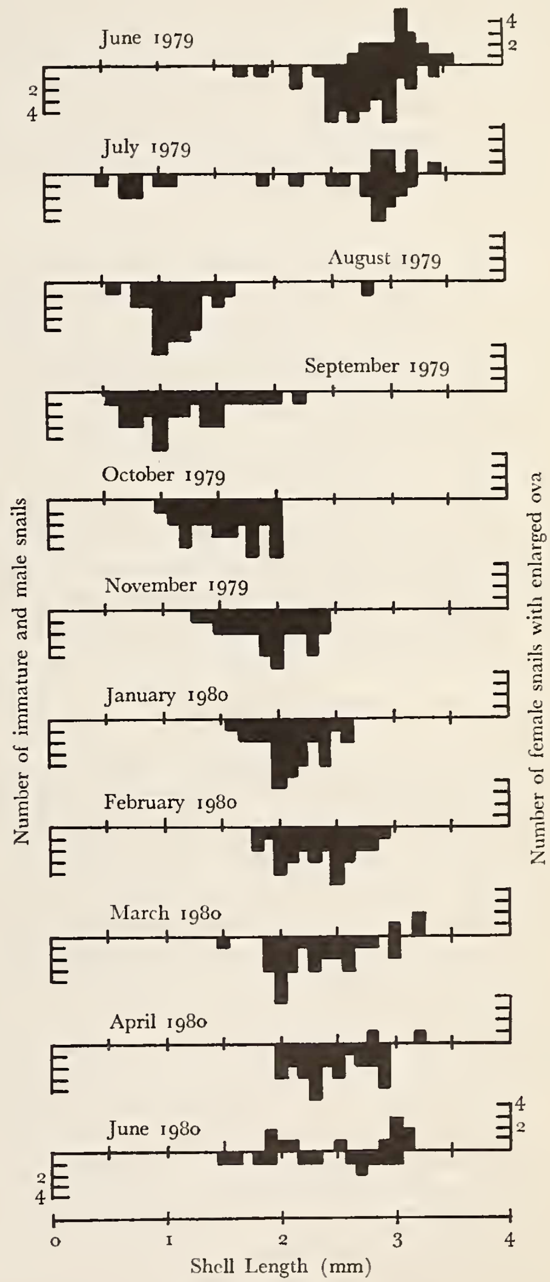

Figure 5

Size-class distribution of the *Tricolia pulloides* population (immature and male snails below horizontal line, ripe females above) in approximately monthly samples from Mussel Point, June 1979 to June 1980 development is quick and easy to follow. Development from egg-laying (fertilization?) to hatching takes only about 7 days at 14° C, and at this temperature most eggs developed normally. However, at room temperature (about 23° C) many eggs failed to develop or developed abnormally. Under intense light and moderate heat, embryos were seen to rotate much more frequently within the egg membrane. At low tide on warm days the egg clutches may sometimes be exposed to increased temperature, possibly affecting mortality and rate of development.

Tricolia pulloides has a relatively direct development and lacks swimming trochophore and veliger stages. In contrast, the British *T. pullus* develops via a free-swimming trochopore larva (LEBOUR, 1937). Direct development, bypassing a pelagic stage, is comparatively uncommon among prosobranchs (WEBBER, 1977), yet it has been documented in the closely related *Tricolia (Hiloa) variabilis* (Pease, 1860)(see WERTZBERGER, 1968 a,b; KAY, 1979). The occurrence of direct development may help account for the somewhat patchy distribution of *T. pulloides* in the intertidal region. Where dispersal is probably mostly by creeping, local populations may develop. Further investigation of this would be desirable.

SUMMARY

1. The diet and reproductive biology of *Tricolia pulloides* was investigated during the period April, 1979 - June, 1980, at Mussel Point, Pacific Grove, California.
2. *Tricolia pulloides* feeds primarily on pennate diatoms grazed as epiphytes from fronds of such red algae as *Rhodoglossum affine* and *Gigartina papillata.* Small particles of detritus and pieces of other epiphytes are probably picked incidentally. Food is found in the gut at both high and low tide.
3. *Tricolia pulloides* shows roughly a 1 : 1 ratio of males and females during the most active reproductive period. Ripe females (averaging 3.14 mm in length) are very significantly larger than the ripe males (averaging 2.62 mm in length) though the smallest females are considerably smaller than the largest males.
4. *Tricolia pulloides* appears to have a life span of about a year. Sexual maturity and reproduction occur at 9 - 11 months.
5. The ovary in ripe females carried up to 206 and averages 56.5 yellow, yolky oocytes about 150 μm in diameter. Eggs are laid in a sinuous ribbon coated with clear jelly, the whole forming a disc-shaped clutch measuring about 2.0 x 2.5 mm, containing up to 104 eggs but averaging 32.5 eggs. Egg clutches are attached to red algal fronds in the midtide zone.
6. Eggs cleave spirally and development is direct. Within 7 days at 14° C they hatch as miniature snails that feed on naviculoid diatoms and cling tenaciously to algal fronds with the aid of mucus threads.
7. All stages of the life history are easily maintained in the laboratory, suggesting this might make a useful experimental animal.

ACKNOWLEDGMENTS

I would like to thank William Magruder and the faculty and staff of Hopkins Marine Station for their unfailing assistance and copious humor throughout this project. My deepest thanks go to Dr. Isabella A. Abbott for her patient aid and expertise in identifying gut contents, and to both Drs. Donald P. Abbott and Isabella A. Abbott for their meticulous advice and foresight. Never could my excitement have been channeled so constructively without their aid.

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